Impacts of Soil Amendment History on Nitrogen Availability from Manure and Fertilizer

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Repeated, long-term additions of organic materials not only increase stocks of mineralizable soil N, but also bring about changes in soil characteristics that influence N dynamics. We conducted an aerobic incubation to explore how soil amendment history affects the transformation and availability of recently added N. Soil was collected from plots under contrasting amended and nonamended soil management systems in a 13-yr cropping systems experiment. Nitrogen source treatments were: no added N (control), NH₄+ fertilizer (Fert), a net mineralizing manure (MManure), and a net immobilizing manure (IManure). Soil NH₄⁺ and NO₃⁻ concentrations were monitored for 282 d. A two-pool, first-order model with fixed rate parameters was fitted to the NO₃- accumulation data. When no N was added, net mineralization in the historically amended soil was twice that in the historically nonamended soil, mostly due to differences in soil total N stocks. When N sources were added, NH₄⁺ consumption, net N mineralization, and estimated N pools were affected by both soil amendment history and N source, with a significant interaction between the two factors. Historically amended soil reduced the availability of recently added N relative to the nonamended soil. This reduction occurred in the active pool (N₁) for MManure and in the slow pool (N₂) for Fert. It appeared to be related to the timing of C availability. Future work modeling N availability should consider soil amendment history not only for its effects on soil N supply capacity, but also for its effects on the availability of recently added N sources.

Abbreviations: Fert, ammonium fertilizer; IManure, net immobilizing manure; MManure, net mineralizing manure; SMB, soil microbial biomass.

Tightening the N cycle by optimizing N use efficiency is fundamental to the design of sustainable agricultural systems (Christensen, 2004). Achieving this goal requires the ability to predict N release from soil organic matter and added N sources (Christensen, 2004; Honeycutt et al., 1991). Soil N dynamics are influenced by environmental factors such as temperature (Andersen and Jensen, 2001; Griffin and Honeycutt, 2000; Honeycutt, 1999) and soil moisture (Griffin et al., 2002; Thomsen et al., 1999). Even under similar environmental conditions, however, N dynamics are also substantially affected by substrate and soil characteristics.

For animal manures, there has been considerable effort to identify chemical characteristics that can be used to refine predictions of N mineralization potential (Cabrera et al., 2005). Most of these studies have focused on the release of plant-available N from manure within a single cropping season. The repeated addition of manure and other organic materials, however, brings about important changes in the soil that can affect N dynamics. Most obvious is the enhancement of the soil organic N pool.

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Only a portion of the organic N in manure is mineralized during the year of application; the remainder accumulates in the soil. While any given application contributes only a small amount to mineralized N in a subsequent year, the combined contributions of organic N from repeated applications can lead to a substantial residual N effect (Eghball et al., 2004; Schröder, 2005), emphasizing the need for consideration of soil amendment history in nutrient management plans (Beauchamp et al., 1986; Feng et al., 2005; Whalen et al., 2001).

In addition to the quantitative increase in the size of the soil organic N pool, repeated long-term application of organic amendments also brings about changes in soil characteristics that could affect N dynamics. As reviewed recently by Cabrera et al. (2005), reduced net N mineralization has been observed repeatedly in finer vs. coarser textured soils following organic N additions, with effects attributed to adsorption of N by clays (Van Veen et al., 1985), greater protection of microbial biomass N (Kuikman et al., 1991; Van Veen et al., 1985), pore-size effects on water availability (Thomsen et al., 1999), and differences in the microbial and grazer communities (Hassink et al., 1994). While organic amendment does not alter soil texture, added organic matter can affect all of the above processes.

Repeated application of organic amendments also adds to the pool of available soil C (Aoyama et al., 1999; Cambardella and Elliott, 1992; Griffin and Porter, 2004; Sommerfeldt et al., 1988) and enhances microbial biomass and activity (Fauci and Dick, 1994; Gunapala and Scow, 1998; Houot and Chaussod, 1995; Witter et al., 1993). Carbon and N cycles are tightly coupled in the soil (Chantigny et al., 2001). The site of this coupling is the soil microbial community, which acts as an important source and sink of both C and N. Research on untilled soils illustrates this

linkage. Barrett and Burke (2000) found a positive linear relationship between soil C concentration and gross rates of mineralization (slope = 0.595) and immobilization (slope = 0.934) in grassland soil, with greater influence on immobilization. Similarly, Hatch et al. (2000) detected a greater increase in immobilization in high-vs. low-C pasture soil 3 mo after a one-time surface application of manure. If these results can be translated to tilled soils, higher gross N transformation rates and retention of added N would be expected in historically amended soil than nonamended soil.

Few studies have investigated the influence of soil amendment history on the mineralization and availability of recently added N substrates. Soil amendment history had no effect on net mineralization of added N (Hadas et al., 1996; Sanchez et al., 2001) or microbial biomass and enzyme activity (Fauci and Dick, 1994) following additions of composted manure and plant residues. These researchers concluded that the response of soil to current N additions far outweighs any differences due to long-term soil management, with no interaction between the two factors. This conclusion may be premature. For instance, both soil amendment history (organic amendments vs. fertilizer) and N source (fertilizer, manure urea, solid manure, and combinations of these), as well as their interaction, significantly affected plant uptake of added N (Langmeier et al., 2002). In their study, where soil C and N differed by only 7 and 15%, respectively, between the contrasting soil treatments, the effect of soil amendment history was an order of magnitude smaller than the effect of N source. A larger soil treatment effect might be expected for soils with more discrepant soil C and N stocks.

The Maine Potato Ecosystem Project provided an ideal opportunity to further explore the potential influence of soil amendment history on N dynamics. Thirteen years of contrasting amended (manure, compost, and green manure) and nonamended soil management systems has resulted in soil with highly divergent C and N stocks. An aerobic incubation of these soils was conducted to: (i) compare the N supplying capacity of historically amended vs. historically nonamended soil; (ii) investigate the effects of soil amendment history on N trans-

formations following addition of fertilizer or manure; and (iii) quantify these possible effects on N pools of differing lability.

MATERIALS AND METHODS Soils and Manures

Soil was collected from the Maine Potato Ecosystem Project, a large, interdisciplinary potato (Solanum tuberosum L.) cropping systems experiment located in Presque Isle, ME, on a gravely, well-drained Caribou loam soil (fine-loamy, mixed, frigid Typic Haplorthod). This experiment has included a comparison of contrasting amended and non-amended soil management systems in the context of 2-yr potato

rotations since 1991. The amended soil management system relied largely on organic sources of nutrients, supplemented with small inputs of inorganic fertilizer. Beef manure and potato compost were applied annually from 1991 to 1993 and semiannually (potato year only) from 1994 to 1998. From 1999 to 2003, manure was applied to both potato and rotation crops, but compost applications were discontinued. The amended soil system also included a pea (Pisum sativum L. subsp. sativum)-oat (Avena sativa L.)-hairy vetch (Vicia villosa Roth) green manure as the rotation crop until 1998, when it was changed to barley (Hordeum vulgare L.) undersown with red clover (Trifolium pratense L.). The nonamended soil management system followed industry standards, including inorganic fertilizers and a barley-red clover rotation crop. The soil treatment factor was in factorial combination with other treatment factors (pest management and potato variety from 1991-1998, and rotation and pest management from 1999-2003) in a split plot design with four replicates. Plot size was 14.6 by 41.0 m. Further details about the soil management systems, other treatment factors, and cultural methods are provided elsewhere (Alyokhin et al., 2005; Gallandt et al., 1998; Mallory and Porter, 2007; Porter and McBurnie, 1996).

Griffin and Porter (2004) reported total, particulate organic matter, and soil microbial biomass (SMB) C and N pools for soil collected in the spring of 1999 from the contrasting soil management systems (Table 1). Identical methods were used to collect and characterize soil in the fall of 2002. Ten-day $\rm CO_2$ evolution rates on both sets of samples were determined as part of the SMB procedure. Soil pH of the 2002 samples was measured in a 1:1 soil/water slurry (Thomas, 1996).

Soil for the aerobic incubation was collected after barley harvest in August 2003 from the four replicate amended and nonamended plots that were in a 2-yr potato—barley rotation and conventional integrated pest management. Six individual soil cores were taken to a depth of 15 cm using an 8-cm-diameter bulb corer. Soil was bulked by soil treatment (i.e., historically amended and historically nonamended), mixed gently, sieved to 2 mm, and stored at 4°C. A 100-g sample of each soil was air dried, from which 5 g was pulverized and analyzed in quadruplicate for total C and N concentration by combustion using a CE Instruments NA2500 Elemental Analyzer (ThermaQuest Italia S.p.A., Rodano, Italy).

Table 1. Characteristics of the historically amended and nonamended soils from the Maine Potato Ecosystem Project, 1999‡ and 2002.

Vasu	Soil history	df S	Soil pH	CO avalution	Carbon			Nitrogen		
Year				CO ₂ evolution	Total	POM§	SMB¶	Total	96 0.62 0 50 0.33 0	SMB
				mg kg ⁻¹ d ⁻¹	g kg			g ⁻¹		
1999	Amended		_	38.6	21.8	8.24	0.48	1.96	0.62	0.12
	Nonamended		-	22.1	16.6	4.02	0.29	1.50	0.33	0.07
2002	Amended		6.3	33.5	33.9	13.68	1.27	2.92	1.02	0.30
	Nonamended		5.5	27.3	17.3	3.79	0.39	1.60	0.31	0.09
						<u>ANOVA</u>				
Source	of variation									
Υe	ear	1	_	NS†	***	***	***	***	***	***
Sc	oil history	1	**	***	***	***	***	**	***	***
Υe	ear × soil history	1	_	NS	***	***	***	***	***	***
C'	V, %		2.2	15.1	5.4	10.4	10.7	5.1	10.0	10.6

^{**} Significant at the 0.01 probability level.

^{***} Significant at the 0.001 probability level.

[†] NS = not significant at the 0.05 probability level.

[‡] From Griffin and Porter (2004).

[§] Particulate organic matter.

[¶] Soil microbial biomass.

Table 2. Characteristics of the net mineralizing (MManure) and net immobilizing (IManure) dairy manures used in the incubation experiment+.

	MManure	IManure
Total C, g kg ⁻¹ ‡	415	451
Neutral detergent fiber (NDF), g kg^{-1}	162	617
Total N, g kg ⁻¹	55.8	14.7
Organic N, g kg ⁻¹	40.0	12.1
NH ₄ +, g kg ⁻¹	15.8	2.6
Total C/N	7.4	30.7
Total C/NH ₄ ⁺	26.3	173.5
NDF/NH ₄ ⁺	10.3	237.3

[†] From Griffin et al. (2005).

Two freeze-dried dairy manures were used in the aerobic incubation, based on N dynamics in a previous incubation experiment (Griffin et al., 2005). The MManure, which resulted in net N mineralization when added to two soils of different textures, had lower total C concentration, higher total N and NH₄⁺ concentrations, and a lower C/N ratio than IManure, which resulted in net immobilization of N (Table 2). While the total C concentration of IManure was only 9% higher than that of MManure, fibrous C concentration (measured as neutral detergent fiber, NDF; Mertens, 2002) was 281% higher. Griffin et al. (2005) found the ratio of NDF to NH₄⁺ to be the best predictor of net nitrification and final NO₃⁻ concentration following manure addition, compared with C/N or other ratios of manure components.

The MManure was obtained from a sample submitted to the Maine Agricultural and Forest Experiment Station Analytical Laboratory and IManure was collected directly from a commercial dairy. The fresh manures were homogenized using a food processor. Subsamples were analyzed for total Kjeldahl N (Kane, 1998) and NH $_4^+$ concentration via distillation with MgO (AOAC Method 973.49). Organic N was estimated as the difference in these values. The remaining manure samples were frozen (–20°C), freeze-dried (–80°C), and ground (2 mm). Ammonium concentrations of the freeze-dried manures used in this study were in the range for fresh manures used by others (Griffin et al., 2005).

Incubation Procedure

Soils were preincubated in the dark for 5 d at 25°C before N additions were made. One hundred and fifty grams of soil (dry-weight equivalent) were added to 250-mL acid-washed, plastic containers and packed to a density of 1.1 g cm $^{-3}$. During the preincubation period, soils were adjusted to a water content of 200 g $\rm H_2O~kg^{-1}$ by either allowing evaporative losses from open containers or adding deionized water.

The MManure (528 mg), IManure (3409 mg), and a fertilizer solution (Fert) (22.3 mg NH₄Cl in 5 mL H₂O) were mixed with samples of each soil on Day 0, an approximate addition rate of 50 mg NH₄+ kg⁻¹ dry soil. This rate is roughly equivalent to 100 kg ha⁻¹ to a depth of 15 cm. A soil-only control treatment was also mixed but no N was added. All treatments were replicated five times. After mixing, a 3-g subsample of the soil (approximately 2.5 g dry-weight equivalent) was placed in a 25-mL centrifuge tube with 25 mL of 2.0 M KCl, shaken for 1 h, and centrifuged (2700 × g for 10 min). The supernatant was filtered (0.45 μ m) and analyzed for NH₄+ and NO₃- colorimetrically on a Lachat Autoanalyzer (Lachat Instruments, Mequon, WI). The remaining soil was repacked to a density of 1.1 g cm⁻³. Deionized water was added to increase the water content to 250 g H₂O kg⁻¹ (47% water-filled pore space, WFPS) and the containers were recapped and returned to the incubator.

Soil $\mathrm{NH_4^+}$ and $\mathrm{NO_3^-}$ concentrations were determined at 1, 3, 7, 14, 28, 56, 112, 171, and 282 d. At each sampling date, the soil was stirred, subsampled, and processed as above, repacked, and returned to the incubator. The soil was aerated by leaving the containers open for 1 h daily for the first 2 wk, and weekly thereafter. Moisture content was maintained by adding deionized water as needed on a weekly basis. Nitrate concentration represented net N mineralization after 3 or 7 d, depending on soil treatment, since $\mathrm{NH_4^+}$ concentrations decreased to and remained near zero for the remainder of the incubation. The proportion of added N that was net mineralized by the end of the incubation was calculated from

$$N_{\text{\tilde{mineralized}}} = (282 \text{dNO}_{3\text{tmt}}^{\text{-}} - 282 \text{dNO}_{3\text{control}}^{\text{-}})/(\text{Nadded})_{\text{tmt}}$$
 [1]

where 282dNO₃⁻ is the NO₃⁻ concentration at 282 d and Nadded is the total N added (as NH₄⁺ and organic N) in the N treatments.

Soil microbial biomass N was estimated at 28 d following the microwave irradiation procedure of Islam and Weil (1998), with the following modifications. After stirring the soil for $\rm NH_4^+$ and $\rm NO_3^-$ sampling, 20-g subsamples (dry-weight equivalent) were removed and placed in small glass beakers, packed to density of 1.1 g cm $^{-3}$, wetted to 70% WFPS, irradiated in a microwave oven to receive 400 kJ kg $^{-1}$ dry soil, stirred, allowed to cool, and then irradiated again. The irradiated soil was inoculated with 1 g untreated soil, repacked to the original density, rewetted to 60% WFPS, and incubated in sealed jars with 5 mL of water in the bottom for 10 d at 25°C. After the incubation period, soil was extracted for $\rm NH_4^+$ and $\rm NO_3^-$ determination as above. Soil microbial biomass N was calculated following Voroney and Paul (1984).

Statistical Analysis

A double exponential model has been found to provide the best description of NO_3^- accumulation in disturbed soil with or without N additions (Benbi and Richter, 2002; Cabrera and Kissel, 1988; Christensen and Olesen, 1998; Deans et al., 1986; Dou et al., 1996; Lindemann and Cardenas, 1984; Wang et al., 2004). This two-pool model allows the separation of N into two conceptual pools: a small, active pool comprised of easily transformed material responsible for an initial rapid phase of NO_3^- accumulation (N_1), and a larger, resistant pool with a slower turnover time (N_2), each described by first-order kinetics. The cumulative amount of accumulated NO_3^- at time t is given as

$$N_{t} = N_{1}[1 - \exp(-k_{1}t)] + N_{2}[1 - \exp(-k_{2}t)]$$
 [2]

where k_1 and k_2 are the rate constants associated with the active and slow N pools.

There are concerns that estimates of N_1 , N_2 , k_1 , and k_2 obtained from fitting all four parameters of the double exponential model simultaneously are highly sensitive to incubation conditions, particularly duration (Benbi and Richter, 2002; Böttcher, 2004; Dou et al., 1996; Wang et al., 2004), and that the rate constants and pool sizes are strongly correlated (Christensen and Olesen, 1998; Wang et al., 2004). For these reasons, some researchers have proposed fixing the rate constants to increase the certainty of the pool size estimates (Christensen and Olesen, 1998; Wang et al., 2003, 2004). This approach focuses on the effects of pool size alone on mineralization.

The double exponential model was fit to NO_3^- accumulation data using both fixed and unfixed rate constants. The values of the fixed rate constants were determined by fitting Eq. [2] to the combined data set of all treatments simultaneously with common k_1 and k_2 parameters but individual N_1 and N_2 for each treatment. The rate constants estimated

[‡] Dry-matter basis.

by this global model were $k_1 = 0.1989 \text{ d}^{-1}$ and $k_2 = 0.0031 \text{ d}^{-1}$ ($R^2 = 0.99$). Model fitting was done with Nonlinear Model (SYSTAT Version 11, SYSTAT Software, 2004) using the least squares loss function and the Marquardt option. Data were first standardized by subtracting the Day 0 soil NO_3^- concentration for each treatment. Curves were fit for each treatment replicate. Extra sums of squares analysis was used to distinguish significantly different curves between soil pairs. The effects of treatment on estimated N_1 and N_2 parameters were analyzed with ANOVA (SYSTAT Version 11, SYSTAT Software, 2004). Parameter means were separated with Fisher's protected LSD procedure, with a Bonferroni adjustment of critical probability values due to multiple tests (Sokal and Rohlf, 1995). The IManure NO_3^- accumulation data could not be fitted with a reasonable model. Instead, repeated measures ANOVA was used to determine the significance of amendment history and sampling date.

RESULTS Soil Properties

A history of soil amendment increased both total C and N concentrations by 31% by 1999, and by 96 and 83%, respectively, by 2002 compared with the historically nonamended treatment (Table 1). The more labile pools of C and N were disproportionately enhanced; particulate organic matter and SMB-C and -N concentrations were two to three times greater in the historically amended soil than in the nonamended soil. Microbial respiration and soil pH were also greater in the historically amended soil than in the nonamended soil. The soil samples used for the incubation were representative of these treatment differences, with total C and N concentrations of 30 and 2.5 g kg $^{-1}$, respectively, for the historically amended treatment and 18 and 1.4 g kg $^{-1}$, respectively, for the historically nonamended treatment.

Soil Nitrogen Mineralization

Net mineralization in the historically amended soil was twice that in the historically nonamended soil during the 282-d incubation when no N sources were added (Fig. 1a). Final soil $\mathrm{NO_3}^-$ accumulated was 168 vs. 84 mg kg $^{-1}$ soil, respectively. Nitrate concentrations reflect net mineralization, as well as nitrification, since $\mathrm{NH_4}$ concentrations were negligible in the control soil throughout the incubation. The proportion of total soil N that was net mineralized during the incubation was also higher in the historically amended soil (6.8%) than the nonamended soil (5.8%; Fig. 1b). Curves fit to the contrasting soil treatments in Fig. 1a and 1b were significantly different (P < 0.001), as determined by extra sums of squares analysis.

Short-Term Nitrogen Dynamics following Nitrogen Additions

Soil $\mathrm{NH_4^+}$ concentrations immediately following the addition of MManure or Fert were equivalent to the target $\mathrm{NH_4^+}$ addition rate (50 mg kg⁻¹) at 0 d and declined at approximately the same rate, reaching zero within 3 to 7 d (Fig. 2). Soil $\mathrm{NH_4^+}$ concentrations in all treatments remained nominal (i.e., <1 mg kg⁻¹ soil) for the remainder of the incubation (data not shown). In the Fert treatment, $\mathrm{NH_4^+}$ consumption and $\mathrm{NO_3^-}$ accumulation appeared to be strongly linked, as the rapid disappearance of $\mathrm{NH_4^+}$ was matched by rapid accumulation of $\mathrm{NO_3^-}$ (Fig. 2 and 3a). Rates of $\mathrm{NO_3^-}$ accumulation slowed once concentrations reached 50 mg kg⁻¹, suggesting complete nitrification of the added fertilizer $\mathrm{NH_4^+}$. In comparison, consumption of $\mathrm{NH_4^+}$ from MManure occurred

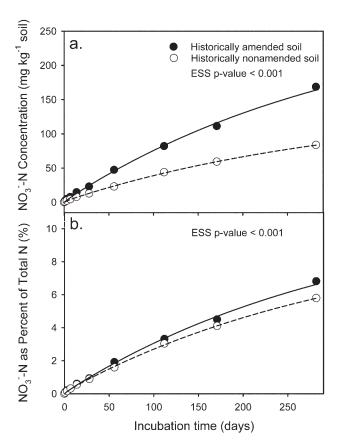


Fig. 1. (a) Nitrate concentration and (b) NO₃⁻ as a percentage of total soil N for historically amended and nonamended soils, fitted with a two-pool, first-order model. Individual data points are the mean of five replicates (n = 5). EES, extra sums of squares comparison of fitted soil treatment curves.

slightly faster than from Fert, but ${\rm NO_3}^-$ accumulated more slowly. Nitrate concentrations in the MManure treatment reached only 31 and 35 mg kg $^{-1}$ in the historically amended and nonamended soils, respectively, by 7 d when ${\rm NH_4}^+$ was depleted (Fig. 2 and 3b).

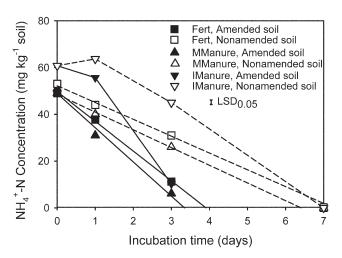


Fig. 2. Ammonium consumption in historically amended and non-amended soils following incorporation of $\mathrm{NH_4}^+$ fertilizer (Fert), net mineralizing manure (MManure), or net immobilizing manure (IManure). Individual data points are the mean of five replicates (n=5). The MManure and Fert data were fitted with linear models ($R^2>0.97$). LSD between all treatments, from a repeated measures ANOVA.

Initial NH₄⁺ concentrations in the IManure treatment exceeded the target application rate by 10 mg kg⁻¹ soil (Fig. 2). Soil NH₄⁺ concentrations showed relatively little change at 1 d, but then decreased rapidly, disappearing by 3 or 7 d. Soil NO₃⁻ concentration in the IManure treatment increased rapidly during this period of NH₄⁺ consumption, as with the other N treatments. Unlike the other treatments, however, soil NO₃⁻ concentrations began to fall once NH₄⁺ was fully consumed, at 3 d for the historically amended treatment and 7 d for the historically nonamended treatment (Fig. 3c).

While N source defined the overall shape of the $\mathrm{NH_4^+}$ consumption and $\mathrm{NO_3^-}$ accumulation curves, amendment history affected N transformation rates. Soil $\mathrm{NH_4^+}$ disappeared and $\mathrm{NO_3^-}$ accumulated more rapidly in the historically amended soil than the nonamended soil for all N sources during the first

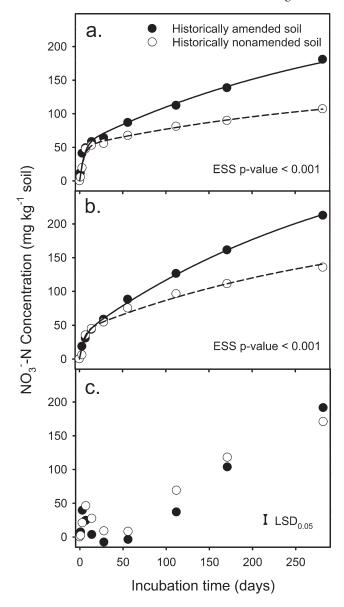


Fig. 3. Nitrate concentration in historically amended and nonamended soils following the incorporation of (a) $\mathrm{NH_4^+}$ fertilizer (Fert), (b) net mineralizing manure (MManure), and (c) net immobilizing manure (IManure). Individual data points are the mean of five replicates (n=5). EES, extra sums of squares comparison of fitted soil treatment curves; LSD between soil treatments, from a repeated measures ANOVA of IManure.

7 d of the incubation. Curves fit to the NO_3^- accumulation data of the contrasting soil treatments were significantly different (P < 0.001 for both Fert and MManure), as determined by extra sums of squares analysis (Fig. 3a and 3b).

Long-Term Nitrogen Dynamics following Nitrogen Additions

The Fert and MManure NO₃⁻ accumulation curves resembled those for the control soils after the initial flush of NO₃⁻, with accumulation occurring more rapidly in the historically amended soil (Fig. 1a, 3a, and 3b). In the IManure treatment, soil NO₃⁻ concentration remained near zero after 3 or 7 d until it began to increase after 56 d. There were relatively small differences between the soil treatments. The negative NO₃⁻ concentrations observed at 28 and 56 d are an artifact of standardizing the data by subtracting Day 0 NO₃⁻ concentrations.

Nitrogen source, soil amendment history, and the interaction of these two factors all affected the proportion of recently added N that was found in the mineral pool at the end of the incubation (Table 3). Within each soil history treatment, Fert was the most available source of N, followed by MManure and IManure. For each N source, less of the recently added N was found in the NO₃⁻ form at 282 d in the historically amended soil than in the historically nonamended soil. The interaction between the treatment factors was due to the difference between soil treatments being smaller for MManure (4.7 units) than for Fert (22.5 units) or IManure (18.8 units).

Soil microbial biomass N, determined at 28 d, was two to three times greater in the historically amended soil than the historically nonamended soil (Table 4). Soil microbial biomass N was also affected by N source, with IManure causing the greatest increase in SMB-N relative to the control and Fert resulting in almost no change.

Table 3. Nitrate pool at the end of the 282-d incubation, expressed as a percentage of N added (NH₄⁺ plus organic N), for the historically amended and nonamended soil treatments.

N source‡	df	Amended	Nonamended
			_ %
Fert		24.5§	47.0
MManure		22.3	27.0
IManure		6.7	25.5
		<u>ANOVA</u>	
Source of variation			
Replicate	4		NSt
Soil history (S)	1		***
N source (N)	2		***
$S \times N$	2		**
LSD(0.05)			0.07
CV, %			21.3

^{**} Significant at the 0.01 probability level.

^{***} Significant at the 0.001 probability level.

[†] NS = not significant at the 0.05 probability level.

[‡]Fert = NH₄ † fertilizer; MManure = net mineralizing manure; IManure = net immobilizing manure.

^{\$} Calculated by dividing control-corrected NO3 $^-$ concentrations at 282 d by the sum of NH₄ $^+$ and organic N added for each N source.

Estimated Nitrogen Pool Sizes

The double exponential model provided good fits for NO₃ accumulation curves of the Fert and MManure treatments, regardless of whether rate constants were fitted or fixed (R² values ranged from 0.98 to >0.99 in both cases). The control treatment could be fit with the two-pool model when rate constants were fixed ($R^2 > 0.99$), but with only a single-pool model when the rate constant was fitted ($R^2 > 0.99$). Fitting all the parameters simultaneously produced more variable parameter estimates than fixing the rate constants, as seen with higher ANOVA CVs (Table 5). Also, correlations between the fitted rate constants and pool sizes were high (-0.62 to -0.86 for N_1 and k_1 ; -0.84 to -0.99 for N_2 and k_2), which makes interpretations of the parameters uncertain (Christensen and Olesen, 1998; Wang et al., 2004). Finally, differences in rate constants are difficult to interpret as they represent only the net rate of change in the size of the NO₃⁻ pool, which is the result of multiple opposing processes and cannot be equated with microbial activity. For these reasons, the remaining discussion of pool sizes refers to the estimates of N_1 and N_2 when the rate constants were fixed (Table 5).

Estimated active and slow pools (N_1 and N_2 , respectively) were significantly affected by both N source and soil amendment history (Table 5). There were also significant interactions between these two factors for both N_1 and N_2 . The size of the active pool (N_1) was minimal in the control treatment; this rapidly available N pool may have been exhausted during the 5-d preincubation period. In the Fert treatment, the size of the N_1 pool was equivalent to the amount of NH_4^+ added and was unaffected by soil amendment history. In contrast, N_1 for the MManure treatment was less than the amount

of manure ${}^{1}NH_{4}^{+}$ added, and was 25% lower in the historically amended soil. Rankings of N_{2} for the N treatments were: MManure > control > Fert. The difference in N_{2} between soil treatments for MManure was equivalent to that for the control (135 and 137 mg kg $^{-1}$, respectively), yet this difference was smaller in the Fert treatment (117 mg kg $^{-1}$). The IManure NO_{3}^{-} concentration data could not be fit with the two-pool model, nor could pool sizes be estimated.

DISCUSSION Amendment History Effects on Soil Nitrogen Availability

Thirteen years of organic amendment application created a soil distinct from its nonamended counterpart, with greatly enhanced soil C and N stocks, especially the more readily available pools of C and N, and increased microbial biomass and activity (Table 1). Historical amendment application also doubled the capacity of the soil to supply N. Nitrate accumulation during the incubation and the estimated size of N₂ in the historically amended soil were twice those of the historically nonamended soil when no N

Table 4. Soil microbial biomass (SMB) N concentration at 28 d after N source treatments were added to the historically amended and nonamended soil treatments.

N Source‡	df	Amended	Nonamended			
		mg kg ⁻¹				
Control		259	77			
Fert		241	72			
MManure		308	129			
IManure		513	285			
	A	<u>anova</u>				
Source of variation						
Replicate	4		NS†			
Soil history (S)	1		***			
N source (N)	3		***			
$S \times N$	3		***			
LSD(0.05), mg kg ⁻¹			9			
CV, %			2.8			

^{***} Significant at the 0.001 probability level.

was added (control treatment; Fig. 1a and Table 5). Repeated, long-term application of manure has consistently been shown to increase the N supplying capacity of soil (Burger and Jackson, 2003; Griffin and Laine, 1983; Hadas et al., 1996; Langmeier et al., 2002), with higher application rates resulting in proportionally more potentially mineralizable N (Whalen et al., 2001). In the present study, total soil N concentrations were 79% higher in the historically amended

Table 5. Estimated active (N_1) and slow (N_2) N pool sizes determined by fitting a double exponential model to NO_3^- accumulation curves resulting from the addition of N sources to the historically amended and nonamended soil treatments (Fig. 3a–3c). Pool sizes were estimated either by (i) allowing rate constants $(k_1$ and k_2) to be fit simultaneously, or (ii) fixing the rate constants at $k_1 = 0.1989 \, \mathrm{d}^{-1}$ and $k_2 = 0.0031 \, \mathrm{d}^{-1}$.

T (14		Rate constants fitted						Rate constants fixed		
Treatment‡	df	N ₁	<i>k</i> ₁	df	N_2	k_2	df	N ₁	N_2	
Control										
Amended		-	-		302.0	0.0025		1.5	277.8	
Nonamended		_	_		131.9	0.0036		1.5	140.8	
Fert										
Amended		47.5	0.5574		250.5	0.0028		53.6	211.3	
Nonamended		50.9	0.2140		93.3	0.0034		51.9	94.4	
MManure										
Amended		34.2	0.1607		288.9	0.0035		32.1	307.9	
Nonamended		43.6	0.1310		117.3	0.0050		41.0	172.5	
<u>ANOVA</u>										
Source of variation										
Replicate	4	NSt	NS	4	NS	NS	4	NS	NS	
Soil history (S)	1	***	***	1	***	***	1	***	***	
N source (N)	1	***	***	2	*	***	2	***	***	
S × N	1	*	***	2	NS	NS	2	***	**	
LSD(0.0125)§, mg kg-	-1	5.7	0.1075		65.6	0.0012		3.1	9.1	
CV, %		6.0	18.8		19.1	17.6		5.9	3.0	

^{*} Significant at the 0.05 probability level.

[†] NS = not significant at the 0.05 probability level.

[‡]Fert = NH₄⁺ fertilizer; MManure = net mineralizing manure; IManure = net immobilizing manure.

^{**} Significant at the 0.01 probability level.

^{***} Significant at the 0.001 probability level.

[†] NS = not significant at the 0.05 probability level.

[‡]Fert = NH4⁺ fertilizer; MManure = net mineralizing manure.

[§]Bonferroni-adjusted LSD for experiment-wise Type I error of 5%.

soil than in the historically nonamended soil. Most of the difference between soil treatments was removed when net mineralized N was expressed as a proportion of total soil N (Fig. 1b). This suggests that the size of the substrate pool was the major determinant of N mineralization, and agrees with findings that total soil N is a good predictor of potentially mineralizable N (Cabrera and Kissel, 1988; Griffin and Laine, 1983; Hadas et al., 1986). Total N did not explain all of the difference in net N mineralization, however. At the end of the incubation, 6.8% of total soil N was net mineralized in the historically amended soil vs. 5.8% in the historically nonamended soil. This difference may reflect the relative enhancement of the more readily available pools of N in the historically amended soil.

Numerous edaphic factors other than N pool size also have been shown to influence N mineralization, largely through their effects on microbial activity. These include soil C content (Barrett and Burke, 2000), pore size and water status (Thomsen et al., 1999), soil pH (Curtin and Wen, 1999; Gordillo and Cabrera, 1997), microbial community composition (Hassink et al., 1994; Hassink, 1994), and grazer communities characteristics (Griffiths et al., 2003; Kuikman et al., 1991). Most of these factors or mechanisms are potential contributors to differences in N mineralization between soils with divergent soil amendment histories. It is possible that differences in soil characteristics such as these played a role in the present study, especially given the measured differences in soil C, pH, microbial biomass, and microbial activity (Table 1). Their combined effects were small, however, compared with the effect of the size of the mineralizable N pool.

Nitrogen Mineralization from Recently Added Nitrogen Sources

Nitrogen from manure became available more slowly than fertilizer N. Despite similar NH₄⁺ inputs and rates of NH₄⁺ consumption for the MManure and Fert treatments (Fig. 2), NO₃⁻ accumulation was slower (Fig. 3) and estimated N₁ was smaller (Table 5) in the MManure treatment. The proportion of recently added N that was found in the mineral pool at the end of the incubation was also smaller in the MManure treatment than in the Fert treatment (Table 3). These results are congruent with findings that manure is a more gradual source of plant-available N than fertilizer (Langmeier et al., 2002; Ma et al., 1999). Whereas the primary transformation of added fertilizer NH₄⁺ is nitrification, immobilization and nitrification are both stimulated by manure additions, with the possibility that immobilized N can later be remineralized via mineralization-immobilization turnover (Jansson and Persson, 1982). Greater SMB-N in MManure than in the control at 28 d provides evidence that immobilization was indeed an important alternative pathway for this treatment, but not for Fert, which showed no such increase (Table 4). Denitrification has recently been shown to be another important alternative sink in aerobic incubations of manureamended soil, with losses of manure NH₄⁺ up to 30% (Calderón et al., 2004). These losses result from the addition of readily available C and N; C stimulates intense microbial activity, which consumes the local O2 supply, and NO3 fuels denitrification in the anoxic microsites (Calderón et al., 2004, 2005). Denitrification most likely did not play an important role in the present study, however, because the soil was aerated daily and stirred periodically during the period of intense microbial activity and O₂ consumption (0–2 wk).

Alternative pathways for recently added N were even more important for IManure than MManure. In this treatment, there was

an initial flush of NO₃⁻ accumulation, presumably from nitrification of the added NH₄+, followed by a steep drop in NO₃- concentration to near zero at 28 d (Fig. 3c). A similar pattern of N availability was observed for this particular manure in a previous incubation experiment and reflects manure with a high concentration of C relative to NH₄⁺ (Griffin et al., 2005). Greater SMB-N in the IManure treatment than the control at 28 d (Table 4) suggests that microbial immobilization was responsible for the drop in NO₃⁻ concentration. Appreciable rates of NO₃⁻ assimilation by microbes have been observed in tilled and untilled soils, and have been associated with C availability (Burger and Jackson, 2003; DeLuca and Keeney, 1995; Schimel, 1986). The IManure added seven times more total C than MManure at the same NH₄⁺ addition rate (10247 vs. 1460 mg C kg⁻¹ soil, respectively) due to its high C/NH_4^+ ratio (Table 2). Additionally, IManure C was substantially more recalcitrant than MManure C, probably becoming available more slowly. Calderón et al. (2005) observed that lower cumulative N2O flux correlated with lower CO₂ flux regardless of manure total C concentration. They hypothesized that slow and gradual sources of C favor immobilization over denitrification, as would a well-aerated soil status. Following the drop to near zero at 28 d in the present study, NO₃concentrations began accumulating in IManure, indicating a shift in the relative importance of ammonification and subsequent nitrification over immobilization.

Net mineralization of organic N was observed for MManure as an increase in $\rm N_1$ + $\rm N_2$ compared with the control (61 and 72 mg kg⁻¹ for the historically amended and nonamended soils, respectively) that was greater than the amount of $\rm NH_4^+$ added (approximately 50 mg kg⁻¹). Organic N can contribute to active, slow, and recalcitrant pools of N (Wander, 2004), while $\rm NH_4^+$ is assumed to be part of the active N pool. The relative contributions of organic N and $\rm NH_4^+$ to $\rm N_1$ and $\rm N_2$ cannot be determined in the present study. The coincidence of $\rm NH_4^+$ disappearance and $\rm NO_3^-$ accumulation during the first 7 d, however, suggests that $\rm NH_4^+$ was the largest contributor to $\rm N_1$. Net mineralization of organic N was probably responsible for the greater $\rm N_2$ in MManure relative to the control.

The Fert treatment resulted in a smaller N_2 pool relative to the control. Reduced NO_3^- concentrations could have occurred from loss of mineral N, suppression of soil N mineralization, or both. Denitrification is not a likely mechanism for lowering NO_3^- levels in the Fert treatment for the reasons mentioned above and because Fert did not introduce a source of readily available C. It is more probable that net mineralization was suppressed by the addition of N fertilizer. Mineralization of organic N is known to decrease progressively with decreasing pH below pH 6 (Adams and Martin, 1984). The pH of the incubation soils started in this range (Table 1) and could have been reduced by nitrification of the added NH_4^+ fertilizer (Brady and Weil, 1996). While a pH effect is a more likely explanation than denitrification, the actual cause of the reduced Fert N_2 relative to the control remains unclear.

Amendment History Effects on Mineralization of Recently Added Nitrogen

Results from the historically amended and nonamended soils indicate that the effects of soil amendment history on mineralization of recently added N can be more important than previously documented. Although the N source treatment factor defined the overall shapes of the NO₃⁻ accumula-

tion curves, soil amendment history also clearly influenced N dynamics. In the short term (0–7 d), initial rates of $\mathrm{NH_4^+}$ disappearance and $\mathrm{NO_3^-}$ accumulation were higher in the historically amended soil than the nonamended soil in almost all cases (Fig. 2 and 3a–3c), presumably due to a larger, more active microbial biomass (Table 1). In the long term, however, historical amendment had the opposite effect, reducing rather than increasing the availability of recently added N. Recovery of added N as $\mathrm{NO_3^-}$ at the end of the incubation was lower in the historically amended soil than in the historically nonamended soil (Table 3), suggesting that immobilization of recently added N was more important in the historically amended soil.

Previous research has found little or no effect of soil amendment history on the availability of current N additions (Hadas et al., 1996; Langmeier et al., 2002; Sanchez et al., 2001). Langmeier et al. (2002) reported a significant effect of soil management (organic vs. mineral fertilizers) on plant uptake of N from mineral and organic N sources, but the soil effect was an order of magnitude smaller than N source effects, and was only observed for organic N sources. In contrast, our results demonstrate that the effects of soil amendment history on the availability of N from organic and inorganic sources can be as important in scale and duration as N source effects. One possible reason why our results do not concur with others is that the historically amended and nonamended soils were far more disparate than the pairs of contrasting soils used in the other studies. For example, total soil C and N concentrations, 67 and 79% higher, respectively, in the historically amended soil than in the historically nonamended soil, differed between soil pairs by only 7 and 15% in Langmeier et al. (2002), by 36 and 30% in Sanchez et al. (2001), and by 61% (reported for total soil N only) in Hadas et al. (1996).

Estimating the active and slow N pools with the double exponential model revealed that, although the historically amended soil reduced the availability of all sources of N, the pools affected were not the same. Historical amendment affected N_1 for MManure and N_2 for Fert (Table 5). One possible explanation involves the relative availability of the different sources of C that could facilitate N immobilization, namely soil and manure. Although soil C was much more abundant in the historically amended soil than the historically nonamended soil, the preincubation period may have depleted both soils of the most readily available C pools. If so, immobilization of Fert NH₄⁺ may have been C limited in the short term, thereby favoring nitrification. With time, however, mineralization of soil organic matter would have liberated soil C, with more becoming available in the historically amended soil, and allowed immobilization in the Fert treatment. This apparent lag time for C availability could explain why historical amendment affected N_2 but not N_1 in the Fert treatment. In the MManure treatment, this lag time for C availability may have been overcome by the addition of labile C in the manure. In this case, the reduction of N₁ in the historically amended soil could be attributed to an interaction of a more active soil microbial community with the added C and NH₄⁺ (Burger and Jackson, 2003), resulting in increased immobilization relative to nitrification (Barrett and Burke, 2000; Hatch et al., 2000).

Although pool sizes were not estimable for IManure, the NO_3^- accumulation results (Fig. 3c) show a reduction in soil

 ${
m NO_3}^-$ concentration in the historically amended treatment relative to the nonamended treatment. This reduction did not occur until after the initial flush of ${
m NO_3}^-$ (after 3 d), suggesting that C availability was delayed in the historically amended soil receiving IManure. In this case, the apparent lag time for C availability was due to recalcitrance of the manure C (Table 2) as well as of the soil C.

Implications of an Amendment History Effect

Two factors determine how N use efficiency might be impacted by the reduced availability of recently added N in a historically amended soil: (i) the magnitude and timing of plant N demand relative to N supply, and (ii) the fate of the recently added N not recovered in the inorganic N pool. Inorganic N in excess of plant demand is susceptible to loss via leaching or denitrification. Creating better coincidence between N supply and plant demand is central to improving N use efficiency and tightening the N cycle (Christensen, 2004). Delaying or reducing N availability from added sources, as occurred in the historically amended soil, may increase synchrony with plant demand and reduce potential N leaching losses (Ma et al., 1999), but may also lead to potentially leachable end-of-season excesses of NO₃⁻ (Schröder, 2005).

The fate of recently added N not recovered in the NO₃ pool of the historically amended soil depends on the mechanism responsible for the soil history effect. It appears that reductions were related to microbial activity and available manure C in the short term (0-7 d), and to available soil and manure C in the longer term. Carbon-enhanced immobilization is a probable mechanism since it is microbiologically driven, dependent on a readily available source of C, and provides an alternative pathway for NH₄⁺. Immobilized NH₄⁺ enters the microbial biomass instead of the NO₃⁻ pool. As mentioned above, denitrification can be another important pathway for manure N (Calderón et al., 2004), although of unlikely importance in the present study. Distinguishing between immobilization and denitrification of recently added N is not necessary for predicting plant-available N during the first growing season after application, but it is critical for estimating the longer term N supply effects (Lindemann and Cardenas, 1984) as well as the environmental impact of manure amendments. While both processes reduce current-season plant-available N, denitrification results in net loss of N from the system to the environment. In contrast, immobilization builds the N supply capacity of the soil, reduces potential N losses via leaching, and thereby increases the overall N efficiency of the agricultural system (Christensen, 2004).

Soil amendment history had the largest impact on soil N mineralization capacity through the accumulation of residual N, but it also altered the dynamics of recently added N. As such, future work to develop and refine predictive models for N availability should include consideration of soil amendment history not only for its effects on the ability of the soil to supply N, but also for its effects on the availability of recently added N sources. Additionally, an understanding of the fate of added N not recovered in the NO₃⁻ pool in historically amended soil, and of how it is influenced by manure and fertilizer characteristics, is clearly needed to predict the long-term availability and the potential environmental impact of N added to these soils.

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